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26 MAR 2003
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26MAR03 E795222-1 C95962
P01/7700 0.00-0306907.7

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
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South Wales
NP10 8QQ

1. Your reference AP 11

2. Patent application number *(The Patent Office will fill in this part)* 26 MAR 2003 0306907.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

ANGIOGENE PHARMACEUTICALS LTD
MAGDALEN CENTRE
OXFORD SCIENCE PARK
OXFORD
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Patents ADP number *(if you know it)* 72444 8003

If the applicant is a corporate body, give the country/state of its incorporation SCOTLAND

GRAY LABORATORY CANCER RESEARCH TRUST
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4. Title of the invention

BIOREDUCTIVELY-ACTIVATED PRODRUGS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Patents ADP number *(if you know it)*

	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

	Number of earlier application	Date of filing *(day / month / year)*
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	20
Claim *(s)*	
Abstract	
Drawing *(s)*	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature P. Wardman Date 26/3/03

12. Name and daytime telephone number of person to contact in the United Kingdom PETER DAVIS 01865 784660

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Notes

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BIOREDUCTIVELY-ACTIVATED PRODRUGS

This invention relates to compounds useful in the treatment of cell proliferation disorders. More particularly the invention relates to a series of compounds that are activated under hypoxic conditions.

Many drugs used in conventional cancer chemotherapy are toxic to growing cancer cells but lack complete specificity. Thus other normal tissues are affected and ensuing side effects limit the dose that can be administered. Therefore the exposure of the cancerous tumour to the compound, and in turn the effectiveness of the therapy, is limited. Recent research has shown promising clinical activity of compounds, such as protein kinase inhibitors, which are cytostatic in their action. However the specificity of such compounds is not complete and side effects arising from action against normal tissues can again limit the effectiveness of therapy. There is a need for drugs that target the tumour more selectively.

Many solid tumours exhibit regions of hypoxia (low oxygen tension). Inadequate blood supply to the central regions of the tumour results in hypoxia that can be chronic or acute. This hypoxia represents a challenge to effective therapy by radiation or by conventional chemotherapy since hypoxic regions are often more resistant to these modalities. It has been suggested, however, that tumour hypoxia can be used to target tumours for drug action (Kennedy, Cancer Res. 1980, *40*, 2356-2360.). One particular method of using the hypoxic regions of tumours for drug targeting is the selective activation of produgs under conditions of low oxygen tension. A concept has been advanced whereby the activity of a cytotoxic compound can be masked by a trigger moiety which, under hypoxic conditions, mediates fragmentation of the masked cytotoxic compound into the active cytotoxic agent (Denny, Lancet Oncol 2000, *1*, 25-9). Compounds attempting to utilize this concept typically consist of the trigger moiety attached, often via a linker moiety, to a cytotoxic moiety (the effector).

Hypoxia is also a feature of the rheumatoid arthritic joint (Rothschild Semin Arthritis Rheum 1982, *12*, 11-31). Cell proliferation is also a feature of the arthritic joint. Systemic antiproliferative drugs (for example methotrexate) are used in the therapy

of rheumatoid arthritis but are limited by side effects. Psoriatic lesions are also characterized by cell proliferation and hypoxia (Dvorak Int Arch Allergy Immunol. 1995, *107*, 233-5.

A number of hypoxic trigger moieties have been disclosed including nitrobenzenes, nitronaphthalenes, nitroimidazoles, nitrofurans, nitrothiophenes, nitropyrroles, nitropyrazoles, benzoquinones, naphthoquinones, indoloquinones and azidobenzenes (for some examples see Naylor, Mini Rev. Med. Chem. 2001 *1*, 17-29; Tercel, J. Med. Chem. 2001, *44*, 3511-3522 and Damen, Bioorg. Med. Chem. 2002, *10*, 71-77).

A number of effector moieties have been utilised in the art including nitrogen mustards, phosphoramide mustards, taxanes, enediynes and indole derivatives (for some examples see Naylor, *loc cit* and Papot, Curr. Med. Chem. Anti Cancer Agents 2002, *2*, 155-185).

Hypoxic triggers joined to effectors via a linking group have been described wherein the linking group consists of a carbonate or carbamate (for some examples see Naylor, *loc cit* and Papot *loc cit*). In these cases it is intended that the intermediate carbonic acid or carbamic acid, formed by the initial hypoxia-driven fragmentation, further fragments to give the active agent.

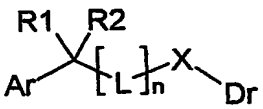
Despite a body of work regarding compounds that break down selectively under low oxygen tensions to release an anticancer agent, no such compound is yet in clinical use. A number of problems have been encountered in the development of such compounds. A lack of stability of the prodrugs towards non-bioreductive processes has been regularly encountered. For example Sartorelli (J Med Chem 1986, *29*, 84-89) has described a series of 5-fluorouracil prodrugs designed to fragment to give 5-fluorouracil under hypoxic conditions but these compounds did not prove useful in this respect due to chemical instability. Borch (J Med Chem 2000, *43*, 3157-3167) has described a series of naphthoquinones designed to release phosphoramide mustards on quinone reduction but these compounds were unstable in cell cytotoxicity assays and released the active agent by mechanisms other than quinone reduction. Similarly the carbonate-linked taxol prodrugs described by Damen (*loc cit*) were

reported to be unstable towards enzymatic hydrolysis in cellular assays, thereby releasing taxol by a non-reductive process. Borch (J Med Chem 2001, *44*, 74-77) has also described a series of hypoxia activated nitroheterocyclic phosphoramidates which were unstable *in vivo*, displaying rapid metabolism and consequent elimination half-lives of only a few minutes. Wilson (J Med Chem 2001, *44*, 3511-3522) has disclosed a series of nitroheteroaryl quaternary salts as bioreductive prodrugs of mechlorethamine but concluded that the compounds were too unstable with regard to non-specific release of mechlorethamine to be of use as bioreductive agents. Thus prodrugs showing improved stability towards non-reductive processes would have advantage.

A further consideration is the rate of release of the active drug under hypoxic conditions. To be effective the bioreductively activated prodrug needs to deliver the drug at a rate which competes with clearance of the prodrug and diffusion of the drug out of the solid tumour. Prodrugs that fragment faster than those in the art, or that fragment more efficiently at oxygen tensions commonly found in solid tumours, would be advantageous.

It is an object of this invention to provide prodrugs that on bioreductive activation break down to release a cytotoxic or cytostatic agent.

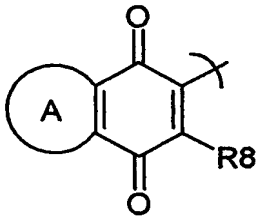
Thus according to one aspect of the invention we provide a compound of formula (1):



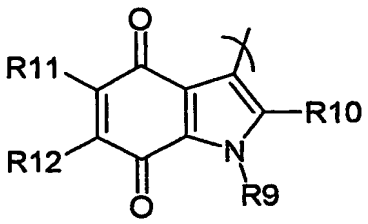
(1)

Wherein:

Ar is a substituted aryl or heteroaryl group bearing at least one nitro or azido group or is a group of formula (2) or (3)



(2)



(3)

R1 and R2, which may be the same or different are independently optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, aryl, COR3 or, together with the intervening carbon atom, form an optionally substituted heterocycloalkyl or carbocyclic ring

L is -OC(O)- or -OP(O)(OR6)-

n is 0 or 1

X is O, S, NR7 or a single covalent bond

R3 is OR4 or NR4R5

R4, R5, R6 and R7 are each independently H or alkyl

R8 is hydrogen, alkoxy or dialkylaminoalkyl

R9 is optionally substituted alkyl

R10 is hydrogen, alkyl, alkoxy or dialkylaminoalkyl

R11 and R12 are independently hydrogen, alkyl, alkoxy, thioalkoxy, amino, alkylamino, dialkylamino, morpholino, piperidino,piperazino or 1-aziridinyl

A is an optionally substituted aryl or heteroaryl ring

Dr is a moiety such that DrXH represents a cytotoxic or cytostatic compound.

As used herein the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl .

An alkenyl group may be for example an olefinic group containing from two to seven carbon atoms for example methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene, s-butylene and t-butylene. An alkynyl group may be for example an ethynyl , propynyl or butynyl group.

Optional substituents which may be present on alkyl, alkenyl or alkynyl groups include one or more substituents selected from halogen, amino, monoalkylamino, dialkylamino, hydroxy, alkoxy, alkylthio, alkylsulphonyl, aryl, heteroaryl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, carboxy, sulphate or phosphate groups. The term "halogen" means fluorine, chlorine, bromine or iodine.

The term aryl means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents examples of which are halogen, optionally substituted alkyl, hydroxy, nitro, azido, cyano, amino and alkoxy.

The term heteroaryl is defined herein as a monocyclic or fused bicyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl , pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups. A heteroaryl group can carry one or more, preferably one to three, substituents examples of which are halogen, optionally substituted alkyl, hydroxy, nitro, azido, cyano, amino and alkoxy.

The term heterocycloalkyl ring includes heterocycloalkyl groups containing 3-6 carbon atoms and one or two oxygen, sulphur or nitrogen atoms. Particular examples of such groups include azetidinyl, pyrrolidinyl, piperidinyl, homopiperidinyl, piperazinyl, homopiperazinyl, morpholinyl or thiomorpholinyl groups. Substituents which may be present on a heterocycloalkyl ring include one or more groups selected from optionally substituted alkyl, halogen, oxo, hydroxy, alkoxy, alkylthio, amino, alkylamino, dialkylamino, carboxy, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylsulphonyl, aminosulphonyl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, sulphate, phosphate and alkylphosphate.

The term carbocyclic ring means a cycloaliphatic group containing 3-10 carbon atoms such as, for example, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. Substituents

which may be present on a carbocyclic ring include one or more groups selected from optionally substituted alkyl, halogen, oxo, hydroxy, alkoxy, alkylthio, amino, alkylamino, dialkylamino, carboxy, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylsulphonyl, aminosulphonyl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, sulphate, phosphate and alkylphosphate.

Cytostatic or cytotoxic compounds represented by DrXH are known or can be determined by standard methods known to those skilled in the art. Such methods include in vitro assays of cell growth using cancer cell lines. Examples of such methods include DNA synthesis assays such as thymidine incorporation assays, protein stain assays such as sulphorhodamine B assays, vital stain assays such as neutral red assays, dye reduction assays such as MTT assays and dye exclusion assays such as trypan blue assays. Appropriate cytotoxic or cytostatic compounds represented by DrXH inhibit cell growth by at least 50% in one or more *in vitro* assays. Thus one skilled in the art can determine the group Dr in formula (1).

More useful values of the groups Dr and X in Formula (1) are those for which the compound DrXH is active in one or more *in vitro* assays of cell growth at concentrations below 1mM.

Most useful values of the groups Dr and X in Formula (1) are those for which the compound DrXH is more potent as a cytotoxic or cytostatic agent, as determined by standard methods, than the corresponding compound of Formula (1).

The moiety Dr may be attached to X such that the group XH in DrXH represents a phenolic or alcoholic hydroxyl group, a carboxylic acid OH group, a thiol group, an anilino group, an alkylanilino group, an amino group or an alkylamino group.

Where n is 0 and X is a single covalent bond, the bond represented by X will be attached to a heterocyclic nitrogen atom in the drug moiety Dr.

Non-limiting examples of DrXH include compounds selected from an anthracyclin antibiotic such as doxorubicin and daunorubicin; an antimetabolite such as 5-fluorouracil, 6- mercaptopurine, 6-thioguanine, cytarabine, gemcitabine, capecitabine, fludarabine, cladribine, trimetrexate and methotrexate; a topoisomerase inhibitor such as an epipodophyllotoxin derivative for example etoposide and teniposide or such as a camptothecin derivative, for example topotecan and SN38; and an inhibitor of mitosis for example a combretastatin derivative such as combretastatin A4, combretastatin A1, and podophyllotoxin, a vinca alkaloid such as vinblastine, vincristine and vinorelbine, a taxane derivative such as paclitaxel and docetaxel, an epothilone derivative such as epothilone B, epothilone D, deoxyepothilone B and BMS 247550, a dolastatin derivative and a cryptophycin derivative. Non-limiting examples of DrH also include inhibitors of protein kinases such as, for example, the anilinoquinazoline inhibitors of protein tyrosine kinases for example gefitinib, erlotinib, ZD6474 and AZD2171.

Where one or more functional groups in compounds of formula (1) are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hrdrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formula (1) may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers which have anticancer activity and mixtures thereof.

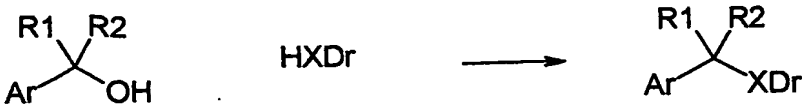
A key and unifying feature of compounds of the present invention is the presence of the substituents R1 and R2. While not limiting on the invention it is believed that the presence of two substituents at this position confers advantage on the compounds by steric and/or electronic effects. For example the increased steric bulk provided by the

two substituents can stabilize the compounds against release of the cytotoxic or cytostatic drug moiety by chemical or enzymatic processes other than the desired bioreductive processes. For another example the absence of a hydrogen atom *alpha* to the aromatic group prevents oxidation at this position; oxidation at this *alpha* position can lead to release of the effector outside of hypoxic regions. For another example the substituents R1 and R2 can extend the range of hypoxic oxygen tensions at which the cytotoxic or cytostatic moiety is released providing increased delivery of the cytotoxic or cytostatic compound to a solid tumour.

It is a further object of this invention to provide methods for the preparation of compounds of Formula (1).

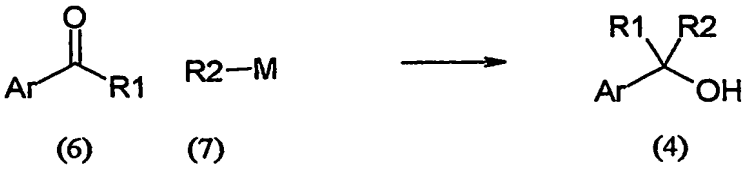
Compounds of Formula (1) may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description, the symbols Ar, R1, R2 Dr, X, n, R7 and R8 when used in the formulae depicted are to be understood to represent those groups described above in relation to Formula (1) unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups that are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Compounds of Formula (1) in which X is O or S and n is 0 can be prepared by Mitsunobu reaction of a tertiary alcohol of formula (4) with a phenol, thiophenol, carboxylic acid, thiocarboxylic acid, alcohol or thiol of formula (5) in a solvent such as an ether solvent, for example tetrahydrofuran, diethyl ether or dioxan or in a solvent such as an aromatic hydrocarbon for example benzene or toluene or in a solvent such as an aprotic solvent for example dimethylformamide, in the presence of a phosphine for example triphenylphosphine or tri-*n*-butylphosphine and in the presence of an azo compound such as diethylazodicarboxylate, diisopropylazodicarboxylate or 1,1'-(azodicarbonyl)dipiperidine at a temperature from about 0°C to about the reflux temperature of the solvent, conveniently at room temperature.

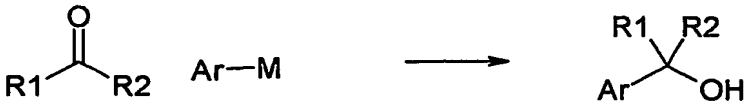


(4) (5)

Alcohols of formula (4) are either known or can be prepared by standard methods apparent to one skilled in the art. Such methods include treatment of a ketone of formula (6) with an organometallic compound of formula (7) in which M represents a metal, metal halide or dialkylmetal, for example, Li, ZnBr, AlR2, MgBr or MgI in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in an aromatic solvent for example benzene or toluene at a temperature of between about -78°C to about the reflux temperature of the solvent, preferably from about 0°C to room temperature. Such methods also include the treatment of a ketone of formula (8) with an organometallic compound of formula (9) in which M represents a metal, metal halide or dialkylmetal, for example, Li, ZnBr, MgBr or MgI or dialkylaluminium in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in an aromatic solvent for example benzene or toluene at a temperature of between about -78°C to about the reflux temperature of the solvent, preferably from about 0°C to room temperature. Where Ar is a substituted aryl or heteroaryl group bearing at least one nitro group such methods also include the aromatic electrophilic nitration of the appropriate aryl substrate with an appropriate nitrating agent at a temperature of between about -78°C and room temperature. Appropriate nitrating agents are, for example, nitric acid in a solvent such as an acid anhydride for example acetic anhydride or in a solvent such as an acid for example sulphuric acid or acetic acid; nitronium tetrafluoroborate in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in a solvent such as acetonitrile or glacial acetic acid or in a solvent such as a chlorinated solvent for example dichloromethane or dinitrogen tetroxide in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in a solvent such as acetonitrile or glacial acetic acid or in a solvent such as a chlorinated solvent for example dichloromethane or in an aromatic solvent for example benzene or toluene.



(6) (7) (4)

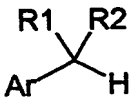


(8) (9) (4)

Compounds of formula (1) in which n = 0 can also be prepared by treatment of a halide of formula (10), in which Hal represents a chlorine, bromine or iodine atom, with a compound of formula (5), in a solvent such as an aprotic solvent such as dimethylformamide or in an ether solvent such as diethyl ether or tetrahydrofuran, or in a ketone solvent such as acetone in the presence of a base such as a metal carbonate for example potassium carbonate or silver(I)carbonate or a base such as a metal hydride for example sodium hydride or potassium hydride, at a temperature of between about -78°C to about the reflux temperature of the solvent preferably between 0° and room temperature.

(10) (5)

Halides of formula (10) are either known or can be prepared by standard methods apparent to one skilled in the art. Such methods include the halogenation of a compound of formula (11) with a halogenating agent such as N-bromosuccinimide, N-chlorosuccinimide or bromine in a solvent such as a chlorinated solvent for example dichloromethane or carbon tetrachloride at a temperature of about between about 0°C and the reflux temperature of the solvent.



(11)

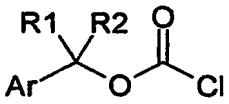
Compounds of formula (1) in which n is 0 and X represents an oxygen atom of a carboxyl group attached to Dr can be prepared by treatment of an alcohol of formula (4) with an acid chloride of formula DrC(O)Cl in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

Compounds of Formula (1) in which X is O, n is 1 and L is -OC(O)- can be prepared by treatment of an alcohol of formula (4) with an acid chloride of formula DrOC(O)Cl in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

Acid chlorides of formula DrOC(O)Cl are either known or can be prepared by standard methods apparent to one skilled in the art . Such methods include treatment of a compound of formula DrOH with phosgene or triphosgene in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane with or without the addition of dimethylformamide at a temperature of around 0°C to room temperature.

Compounds of Formula (1) in which X is NH, n is 1 and L is -OC(O)- can be prepared by treatment of an alcohol of formula (4) with an isocyanate of formula DrNCO in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

Compounds of formula (1) in which X is NR7, n is 1 and L is -OC(O)- can be prepared by treatment of a chloroformate of formula (12) with a compound of the formula DrNHR7 in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.



(12)

Compounds of formula (1) in which n is 1 and L is -OP(O)(OR6)- can be prepared by treatment of an alcohol of formula (4) with a compound of the formula ClP(O)(OR6)XDr in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

Compounds of formula (1) can also be synthesized from other compounds of formula (1) by the application of standard methods, including substitution reactions, functional group transformations, bond-forming reactions and cyclisations known in the art.

Preparation of a compound of Formula (1) as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, vincristine, vinorelbine, paclitaxel and

docetaxel; alkylating agents, for example cisplatin, carboplatin, oxaliplatin, nitrogen mustard, melphalan, chlorambucil, busulphan and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside, gemcitabine, capecitabine, methotrexate and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example aspariginase; topoisomerase inhibitors for example etoposide, teniposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab and trastuzumab; receptor tyrosine kinase inhibitors for example gefitinib, and erlotinib; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 10mg/kg.

According to a further aspect of the invention there is provided a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, for use as a medicament.

According to a further aspect of the invention there is provided the use of a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for use in the therapy of a warm-blooded animal, for example a human, suffering from a proliferative disease for example cancer.

The ability of compounds of the invention to release cytotoxic or cytostatic agents selectively under hypoxic conditions can be assessed by using, for example, one or more of the procedures set out below:

Radiolysis

In the hypoxic environments of solid tumours, prodrugs can be reduced by one-electron processes that are inhibited in the normoxic environments of normal tissues. Radiolysis demonstrates the ability of bioreductively-activated prodrugs to release the active drug after one-electron reduction. Compounds were dissolved in an isopropanol/water mixture (50:50) at a concentration of 50μM or below. Solutions, in gas-tight syringes, were saturated with nitrous oxide before irradiation in a ^{60}Co source at a dose rate of 3.9Gy min^{-1} (as determined by Fricke dosimetry: H. Fricke and E.J. Hart, "Chemical Dosimetry" in Radiation Dosimetry Vol. 2 (F.H. Attrix and W. C. Roesch. Eds.), pp 167-239. Academic Press New York, 1966.). Solutions were analysed for released drug by HPLC. In this test the compound of Example 1 produced combretastatin A4 with a radiation chemical yield (G-value) of 0.36μmol.J^{-1}. The compound of Example 4 produced 6-mercaptopurine with a radiation chemical yield (G-value) for drug release of 0.40μmol.J^{-1}.

Metabolism in tumour homogenates

Useful bioreductive prodrugs can be shown to release the active drug selectively under conditions of low oxygen in the presence of tumour homogenate in this assay. Freshly-excised CaNT tumours (approximately 0.5 to 1g) were homogenised in 15 ml of ice-cold 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4. The homogenates were centrifuged at 1000 RPM for 10 min and the supernatants stored on ice. The metabolism of 5 μmol dm^{-3} prodrug in air and N_2 was performed with 0.5 ml tumour homogenate (~ 3 mg of protein by Bradford assay) with 100 μmol dm^{-3} NADPH in 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4 incubated at 37°C. Samples (60 μl) were taken at regular intervals and added to an equivalent volume of acetonitrile, then mixed and centrifuged at 14, 300 RPM for 2 min prior to product analysis by HPLC. In this test the compound of Example 1 produced combretastatin A4 at a rate of 71 nmol. min^{-1} .mg $protein^{-1}$ under nitrogen but only 0.2 nmol. min^{-1} .mg $protein^{-1}$ under air (a differential of 355). In contrast the compound 1-(4-methoxy-3-(5-nitrothiene-2-yl)methoxy)phenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene, lacking the key features of the invention had a differential of only 8.

Cellular Cytotoxicity

In a preferred embodiment of the invention the compounds of formula (1) will be less potent as cytotoxic or cytostatic agents than the corresponding cytotoxic or cytostatic compounds of formula DrXH which are released under hypoxic conditions. The cytotoxic or cytostatic properties of compounds of formula (1) and compounds of formula DrXH can be assessed for example, by use, for example, of this assay. The Celltiter 96® Aq_{ueous} One Solution Cell Proliferation Assay kit (Promega Corporation, USA) which is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays was used. In this assay the MTS tetrazolium compound (Owen's Reagent) is bioreduced by viable cells into a coloured formazan product which is soluble in tissue culture medium and can be measured by recording absorbance at 490 nm with a 96 well plate reader. A549 cells were seeded in Eagles Minimum Essential Medium supplemented with 10% foetal calf serum and non-essential amino acids at 10^3 cell per well on a 96 well plate and allowed to attach for 24 h. Compounds were dissolved in DMSO and diluted with cell culture medium

before addition. The cells were exposed to test compound (0 to 2 μmol dm^{-3}) for 6 h then incubated for a further 72 h. The MTS reagent was added to each well, left for 4 h, then the absorbance measured at 490 nm with a 96 well plate reader. In this assay the compound of Example 1 had no activity at concentrations up to 2μM whereas combretastatin A4 reduced cell numbers to 50% of control at a concentration of around 250nM.

Metabolism in Liver Homogenates

Metabolic stability of the compounds and unfavorable release of the drug by oxic liver can be assessed by using, for example, this assay. Freshly-excised mouse liver (approximately 1g) was homogenised in 15 ml of ice-cold 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4. The homogenates were centrifuged at 1000 RPM for 10 min and the supernatants stored on ice. The metabolism of 5 μmol dm^{-3} prodrug in air was performed with 0.5 ml liver homogenate (~ 4 mg of protein by Bradford assay) with 100 μmol dm^{-3} NADPH in 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4 incubated at 37°C. Samples (60 μl) were taken at regular intervals and added to an equivalent volume of acetonitrile, then mixed and centrifuged at 14, 300 RPM for 2 min prior to product analysis by HPLC. In this test the compound of Example 1 produced combretastatin A4 at a rate of only 0.5 nmol. min^{-1} .mg $protein^{-1}$. In contrast the corresponding compound 1-(4-methoxy-3-(5-nitrothiene-2-yl)methoxy)phenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene, lacking the key features of the invention produced combretastatin at a greater rate of 3 nmol. min^{-1} .mg $protein^{-1}$.

The invention is illustrated by the following non-limiting Examples in which, unless otherwise stated:

DMF means dimethylformamide
THF means tetrahydrofuran
MeOH means methyl alcohol

EtOAc means ethyl acetate

DCM means dichloromethane

TLC means thin-layer chromatography

MeCN means acetonitrile

TFA means trifluoroacetic acid

Example 1

1-(4-Methoxy-3-(2-(5-nitrothiophen-2-yl)propan-2-yl)oxyphenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene

1-Methyl-1-(5-nitrothiophen-2-yl)ethanol (200 mg, 1.07 mmol) was dissolved in benzene (2.5 ml) together with combretastatin A4 (320 mg, 1 mmol) and 1,1-(azodicarbonyl)dipiperidine (ADDP, 250 mg, 1 mmol) and the solution maintained under argon with stirring. Tributylphosphine (200 mg, 1 mmol, dissolved in benzene (0.5 ml)) was then added via syringe and under argon. The solution was stirred for 24 h at 20°C and then partitioned with EtOAc/water (100 ml) and the organic layer washed with brine (50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography on silica gel (33% EtOAc/hexane) and then on a second silica column (DCM) to give a pale yellow oil (150 mg, 31%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 5 Hz, 1H), 7.05 (d, J = 5 Hz, 1H), 6.86 (d, J = 5 Hz, 1H), 6.81 (s, 1H), 6.74 (s, 1H), 6.475 (d, J = 5 Hz, 4H), 3.89 (s, 3H), 3.85 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H) ppm. MS (m/z, %) 485 (M^+, 4.3 %), 316 (100 %), 301 (56 %). LC-RT 4.34 minutes (100 % MeCN).

Example 2

1-(4-Methoxy-3-(2-(4-nitrophenyl)propan-2-yl)oxyphenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene

Sodium hydride (9 mg, 0.22 mmol) was added to combretastatin A4 (60 mg, 0.19 mmol) in DMF (0.2mL). To this was added 2-bromo-2-(4-nitro)phenylpropane (54 mg, 0.22 mmol) in DMF (0.2 mL) and the reaction was stirred for 72 h. The reaction

mixture was partitioned (EtOAc and brine), the aqueous phase was extracted (EtOAc), the organic phases were combined then dried ($MgSO_4$) and evaporated. Preparative TLC, using 10% EtOAc/hexane as solvent, yielded the product as a wax (8 mg, 9%); TLC R_f=0.15, 10% EtOAc/hexane; LC-RT 4.14 minutes (100% MeCN). MS (m/z, %) 479 (M^+, 15 %), 316 (100 %), 301 (66 %), 163 (15 %), 149 (9 %), 133 (40 %).

Example 3

9-(7,8-Dihydroxy-2-methyl-hexahydro-pyrano[3,2-d][1,3]-dioxin-6-yloxy)-5-{3,5-dimethoxy-4-[1-methyl-1-(4-nitrophenyl)-ethoxy]-phenyl}-5,8,8a,9-tetrahydro-5aH-furo[3',4':6,7]naphtho[2,3-d][1,3]dioxol-6-one

Sodium hydride (40 mg, 0.84 mmol) was added to a mixture of etoposide (144 mg, 0.56 mmol), 2-bromo-2-(4-nitro)phenylpropane (204 mg, 0.84 mmol) in DMF (0.5 mL) and the reaction was stirred for 72 h. The reaction mixture was partitioned (EtOAc and brine), the aqueous phase was extracted (EtOAc), the organic phases were combined then dried ($MgSO_4$) and evaporated. Preparative TLC, using EtOAc as solvent, and then preparative HPLC afforded the product as a wax (8 mg, 2 %); TLC R_f=0.7, EtOAc. LC-RT 6.29 minutes (TFA 50-100 %). MS (m/z, %) 663 (1 %), 401 (1 %), 398 (1 %), 382 (5 %), 353 (1 %), 324 (3 %), 163 (100 %), 150 (20 %), 133 (80 %).

Example 4

6-(2-(4-nitrophenyl)propan-2-ylsulfanyl)-9H-purine

Sodium hydride (80 mg, 1.96 mmol) was added to 6-mercaptopurine (308 mg, 1.88 mmol) in DMF (2 mL). To this was added 2-bromo-2-(4-nitro)phenylpropane (400 mg, 0.98 mmol) in DMF (2 mL) and the reaction was stirred for 24 h. The reaction mixture was partitioned (EtOAc and brine), the aqueous phase was extracted (EtOAc), the organic phases were combined then washed (water then brine), dried ($MgSO_4$) and evaporated. Flash chromatography, eluting with 50 % and 75 % EtOAc/hexane then 100% EtOAc, afforded a fluffy white solid (101 mg, 33 %); TLC R_f=0.48, EtOAc; mp

206-208 °C; LC-RT 4.2 minutes (TFA 50-100 %). MS (m/z, %) 315 (M^+, 8 %), 163 (40 %), 152 (100 %), 133 (25 %), 125 (20 %).

Example 5

1-(4-Methoxy-3-(1-methyl-4-(5-nitrothien-2-yl)piperidin-4-yl)oxycarbonyloxy)phenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene

Phosgene (0.1 mL, 0.20 mmol, 20% solution in toluene) was added to DCM (0.5 mL) at 0°C. To this was added combretastatin A4 (56 mg, 0.18 mmol) in DCM (0.5 mL), followed after 1 hour by triethylamine (28 μL, 0.20 mmol). After 6 hours, the reaction mixture was added drop-wise to a cooled (0°C) solution of 4-hydroxy-1-methyl-4-(5-nitrothien-2-yl)piperidine (44 mg, 0.18 mmol), pyridine (15 μL, 0.18 mmol), DCM (1 mL) and DMF (1 mL). The reaction mixture was allowed to reach ambient temperature and stirred for a further 2 hours. The brown solution was partitioned (EtOAc, brine), aqueous phase extracted (EtOAc), organic phase washed (H_2O, brine), dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography, eluting with 50% EtOAc/hexane, 100% EtOAc then 50% MeOH/EtOAc, furnished the desired product as an orange-brown wax (39 mg, 37 %). R_f=0.34 (50% MeOH/EtOAc); ^1H NMR (500MHz, $CDCl_3$) δ 7.87 (d, 1H, J=5.0Hz, Ar-H), 7.18 (d, 1H, J=5.0Hz, Ar-H), 7.12 (s, 1H, Ar-H), 7.10 (s, 1H, J=5.0Hz, Ar-H), 6.88 (d, 1H, J=5.0Hz, CH), 6.53 (s, 2H, Ar-H), 6.50 (d, 2H, J=5.0Hz, Ar-H, CH), 3.87 (s, 3H, O-CH_3), 3.82 (s, 3H, O-CH_3), 3.74 (s, 6H, O-CH_3), 2.82 (bd, 2H, J=15.0Hz, CH_2), 2.68 (bd, 2H, J=15.0Hz, CH_2), 2.52 (bt, 2H, J=10.0Hz, CH_2), 2.42 (s, 2H, N-CH_3), 2.25 (bt, 2H, J=10.0Hz, CH_2) ppm; LC-RT 5.14 minutes (TFA 50-100%); MS (m/z, %) 584 (M^+, 1 %), 316 (33 %), 301 (40 %), 225 (100 %).

The 4-hydroxy-1-methyl-4-(5-nitrothien-2-yl)-piperidine used as starting material in the above preparation was prepared as follows:

n-Butyllithium (14 mL, 22.4 mmol) was added to a solution of *N,N*-diisopropylamine (2.26 g, 22.4 mmol) in THF (80 mL) at –78°C. After 5 minutes, a solution of 2-nitrothiophene (2.47 g, 19.18 mmol) in THF (10 mL) was added drop-wise. After a further 5 minutes, a solution of 1-methyl-piperidin-4-one (2.53 g, 22.4 mmol) in THF

(10 mL) was added and the reaction mixture stirred for a further 1 hour. The reaction was quenched with saturated $NH_4Cl_{(aq)}$ and concentrated hydrochloric acid (2 mL) then allowed to reach ambient temperature. The reaction mixture was partitioned (EtOAc, H_2O), aqueous phase extracted (EtOAc), neutralised (saturated $NaHCO_{3(aq)}$) then re-extracted (EtOAc). The organic phase was then washed (H_2O, brine), dried ($MgSO_4$) and concentrated *in vacuo* to a brown oil. Flash chromatography, eluting with EtOAc, 50% MeOH/EtOAc and then 100% MeOH, afforded the desired product as a creamy brown solid (572 mg, 12 %), mp 156-157°C; ^{1}H NMR (60MHz, $CDCl_3$) δ 7.81 (d, 1H, J=4.2Hz, Ar-H), 6.91 (d, 1H, J=4.2Hz, Ar-H), 2.68 (s, 3H, N-CH3), 2.33-1.94 (m, 8H, CH2) ppm; LC-RT 2.97 minutes (TFA 20-50%); MS (m/z, %) 242 (M^+, 100 %), 224 (50 %), 197 (29 %).

Example 6

1-(4-Methoxy-3-(2-(1-methyl-5-nitroimidazol-2-yl)propan-2-yl)oxyphenyl-2-(3,4,5-trimethoxy)phenyl-*Z*-ethene

5-(1-Hydroxy-1-methylethyl)-1-methyl-2-nitro-1H-imidazole (10 mg, 0.054 mmol) was dissolved in THF (1.5 mL) together with triphenylphosphine (42 mg, 0.16 mmol) and combretastatin A4 (51 mg, 0.16 mmol). Diethylazodicarboxylate (28 mg, 0.16 mmol) was then added and the solution stirred for 18 h at room temperature. A further amount of 5-(1-hydroxy-1-methylethyl)-1-methyl-2-nitro-1H-imidazole (10 mg, 0.054 mmol) was then added and after a further 18 h the solution was applied directly to a silica column and eluted with 25 % EtOAc/hexane to give the title compound as a yellow gum (30 mg, 15 %). LC-RT 6.55 minutes (TFA 50-100%); MS (m/z, %) 484 (M^+, 6 %), 438 (6 %), 317 (100 %), 302 (54 %), 170 (16 %).